AUTHORITY: 21 U.S.C. 357.

Subpart A—Bulk Drugs

§ 450.10a Sterile bleomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile bleomycin sulfate is the amorphous sulfate salt of bleomycin. Bleomycin has been separated into several similar glyco-peptide molecules. It is a cream-colored powder that is so purified and dried that:

(i) Its potency is not less than 1.5 units and not more than 2.0 units of bleomycin per milligram. If it is packaged for dispensing, the content of the ampoule or vial is not less than 90 percent and not more than 120 percent of the number of units of bleomycin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 6.0 percent.

(vii) Its pH in an aqueous solution containing 10 units per milliliter is not less than 4.5 and not more than 6.0.

(viii) Its copper content is not greater than 0.1 percent.

(ix) Its content of various bleomycins is as follows: Bleomycin A_2 is not less than 55 percent and not more than 70 percent; bleomycin B_2 is not less than 25 percent and not more than 32 percent; bleomycin B_4 is not more than 1 percent. Bleomycins A_2 and B_2 should comprise not less than 85 percent of the total bleomycins.

(x) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, copper, content of various bleomycins, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: A minimum of 20 immediate containers.

(*b*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 7.0 (solution 16), to provide a stock solution of convenient concentration; if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents. Dilute the sample thus obtained with solution 16 to provide a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 16 to the reference concentration of 0.04 unit of activity per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use the entire contents of each of the immediate containers tested.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 0.5 unit of bleomycin per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter, using the total contents of 2 or 3 vials.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 units per milliliter.

(8) *Copper content*—(i) *Reagents.* Dissolve 10 milligrams of zinc dibenzyldithiocarbamate in 100 milliliters of carbon tetrachloride.

(ii) *Preparation of standard copper solution.* Accurately weigh 1.965 grams of cupric sulfate pentahydrate and transfer to a 1-liter volumetric flask. Dissolve the material in 0.1*N* hydrochloric acid, dilute to volume with 0.1*N* hydrochloric acid and mix well. Transfer 3 milliliters of this stock solution to a 1-liter volumetric flask, dilute to volume with 0.1*N* hydrochloric acid, and mix well. This standard copper solution contains 0.0015 milligram of copper per

milliliter. Transfer 10 milliliters of the standard copper solution to a 60-milliliter separatory funnel.

(iii) *Preparation of the sample.* Accurately weigh approximately 15 milligrams of sample into a 60-milliliter separatory funnel. Dissolve the sample in 10 milliliters of 0.1*N* hydrochloric acid.

(iv) *Procedure.* To the separatory funnels containing the sample solution and standard copper solution, add 10 milliliters of the zinc dibenzyldithiocarbamate solution and shake the funnels vigorously for 1 minute. Allow the phases to separate. Filter the carbon tetrachloride phase (lower phase) through 1 gram of anhydrous sodium sulfate to remove excess water. Using a suitable spectrophotometer equipped with 1-centimeter cells, and carbon tetrachloride as a blank, measure the absorbance of the standard copper solution and the sample solution at 435 nanometers. Calculate the percent copper as follows:

$$\text{Percent copper} = \frac{\text{Absorbance of sample solution} \times 1.5}{\text{Absorbance of standard copper solution} \times \text{Sample weight in milligrams}}$$

(9) *Content of various bleomycin fractions.* Proceed as directed in § 436.339 of this chapter.

(10) *Identity test.* Proceed as directed in § 436.211 of this chapter, using the method described in paragraph (b)(1) of that section, using a 1 percent mixture.

[40 FR 52005, Nov. 7, 1975; 40 FR 53998, Nov. 20, 1975, as amended at 46 FR 60568, Dec. 11, 1981; 48 FR 51913, Nov. 15, 1983; 50 FR 19920, May 13, 1985]

§ 450.20 Dactinomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dactinomycin is a bright-red compound that is so purified and dried that:

(i) Its dactinomycin content is not less than 900 micrograms of dactinomycin per milligram, calculated on an anhydrous basis.

(ii) Its loss on drying is not more than 15 percent.

(iii) Its absorptivity at 445 nanometers is not less than 0.95 and not more than 1.03 times that of the dactinomycin working standard at the same wavelength. Its absorbance at 240 nanometers is not less than 1.3 and not more than 1.5 times its absorbance at 445 nanometers.

(iv) It is crystalline.

(v) It passes the identity test for dactinomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter, and in addition each package shall bear on its label the statement ''Protect from light and excessive heat.''

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for dactinomycin content, loss on drying, absorptivity, crystallinity, and identity.

(ii) Samples required: 16 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay.* Dactinomycin is toxic and corrosive. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood, while wearing rubber gloves. Avoid inhaling fine particles of the powder. Do not pipette by mouth. If any of the substance contacts the skin, wash copiously with soap and water. Dispose of all waste material by dilution with large volumes of trisodium phosphate solution.

(1) *Dactinomycin content.* Proceed as directed in § 436.331 of this chapter, preparing the sample and calculating the dactinomycin content as follows:

(i) *Preparation of sample solution.* Accurately weigh a sufficient amount of the sample to obtain a solution containing approximately 0.25 milligram per milliliter of dactinomycin in mobile phase.